

# PATENT SPECIFICATION

(11) 1290 141

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## NO DRAWINGS

- (21) Application No. 26115/68 (22) Filed 31 May 1968  
 (23) Complete Specification filed 29 Aug. 1969  
 (45) Complete Specification published 20 Sept. 1972  
 (51) International Classification A61K 23/00//C07C 103/52 C08G  
 20/08 20/38  
 (52) Index at acceptance  
 A5B 768  
 C2C 20Y 30Y 321 32Y 342 34Y 366 367 576 640 64X  
 738 KZ  
 C3R 22D3A 22N1A 22PX 22T2  
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## (54) VACCINE ADJUVANTS

- (71) We, THE WELLCOME FOUNDATION LIMITED, of 183—193 Euston Road, London, N.W.1., a company incorporated in England; do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—
- 10 This invention relates to vaccines and the preparation thereof.
- It is often desired to enhance the ability of the antigenic material in a vaccine to induce the desired immune response. This may be achieved by use of a vaccine adjuvant and, indeed, with many poorly antigenic materials the success of vaccination depends on the presence of a suitable adjuvant in the vaccine. Thus a vaccine adjuvant may be desirable for any of the following reasons: some antigens are only weakly immunising and fail to produce protective levels of immunity without adjuvants; some antigens are too reactive at the high doses which are necessary if no adjuvant is present; adjuvants may increase the potency of antigens in such a way as to allow fewer doses of vaccine to achieve the required levels of protection; where a single adjuvant is not adequate, an additional adjuvant may allow the total dose to be reduced and undesirable reactions to be minimised.
- It has now been found that certain base-rich peptides consisting of a chain of amino acid molecules linked together by peptide linkages show activity as vaccine adjuvants when tested with a number of antigens. These peptides, are synthetically produced in most cases.
- Thus, according to the present invention, in one aspect, there is provided a vaccine containing non-pathogenic antigenic material together with a base-rich peptide in which at least 50% of the residues have free amino groups, which is pre-
- sent in an amount sufficient to produce an adjuvant effect. To illustrate the wide range of molecular weights over which the peptides of the invention are active, poly-L-lysine was found to be active when of chain length 5, 10, 15 or 30 units, and a poly - L - lysine of molecular weight 100,000 was also found to be active. Usually, though, the highest activity is shown by peptides having molecular weights in the range 6,000 to 50,000. Other noteworthy examples of such peptides are polyornithine, polyarginine and polydiaminobutyric acid.
- Each of the peptides specifically mentioned above has one free amino group on each amino acid residue, i.e. 100% of the amino acid residues contain such free amino groups. However, useful vaccine adjuvant activity is not restricted to such peptides. Thus, useful activity is shown by peptides in which only a proportion of the amino acid residues have free amino groups, for example those in which 50% to 75% of the residues have free amino groups.
- The peptides in the vaccines of the present invention possess certain advantages as vaccine adjuvants. For instance, the adjuvant effect is obtained on injecting simple mixtures of the peptide and antigen, without any attempt at chemical coupling. Also, most immunological adjuvants produce at the site of injection a chronic granuloma. However, the lesions produced by active doses of the peptides are mild and transient, and of little consequence in the context of their use as vaccine adjuvants. Thus, the high activity and low local reactivity of the peptides of the present invention, coupled with the fact that they are potentially metabolizable, make them of special interest, particularly for human use.
- In the vaccines of the present invention, the peptides have been found to be especially active when tested with diphtheria toxoid. The effect of the peptide is to raise the antibody

[Price 25p]

response to a given dose of toxoid many times above the control level. A similar effect has also been observed with tetanus toxoid. The adjuvant activity of the polyamino acids is not, however, restricted to simple diphtheria and tetanus toxoids but is manifest also, for example, with lysine diphtheria toxoids and tetanus toxoid adsorbed onto aluminium hydroxide, both of which are highly antigenic in their own right.

The peptides of the invention also exhibit adjuvant activity when tested with *Cl. welchii* epsilon toxoid and with lysozyme.

In addition, the present invention is particularly applicable to vaccines of the following:

- a) Viral (human):
  - Poliomyelitis
  - Rabies
  - Smallpox (vaccinia)
- Viral (veterinary):
  - Newcastle Disease
  - Avian Infectious Bronchitis
  - Avian Encephalomyelitis
  - Calf Pneumonia
  - Louping ill
  - Ovine Enzootic Abortion
  - Foot and Mouth Disease
  - Equine Influenza
  - Rinderpest
  - Contagious Canine Hepatitis
- b) Bacterial
  - Staphylococcus spp.
  - Streptococcus spp.
  - Corynebacterium spp.
  - Bacillus anthracis
  - Enterobacteria (esp. Salmonella spp. and Escherichia coli)
  - Vibrio (esp. cholerae)
  - Pasteurella spp.
  - Erysipelothrix spp.
  - Listeria spp.
  - Leptospira spp.
- c) Protozoal
  - Leishmania spp.
  - Anaplasma spp.
  - Babesia spp.
  - Toxoplasma spp.
- d) Metazoan
  - Parasitic: Dictiocaulus spp.

Such vaccines may consist of live (e.g. anthrax) or killed organisms, whole or disrupted, or of preparations of natural toxins (e.g. diphtheria, tetanus or *Cl. welchii* toxoids) or products of the organism, or of preparations of extracts of the organisms, alone or in combination with one another.

The peptides in the vaccines of the present invention are conveniently used in the form

of solutions, dispersions or suspensions in a liquid carrier. An adjuvant solution may, for example, be prepared by dissolving in the aqueous diluent used for diluting the antigen. The adjuvant is conveniently incorporated in the vaccine before the latter is distributed into containers, although the adjuvant may be provided in a separate container for mixing with the antigenic material when the vaccine is required for use in immunising the recipient.

The vaccine adjuvant composition so produced is mixed with antigenic material to form a vaccine preparation. The relative amounts needed of the adjuvant and the antigenic material depend on the immunological characteristics of the antigen, and the total amounts of each needed per dose may also depend to some extent on the species and size of the recipient as well as the nature of the infection or intoxication to be immunised against. In small animals such as mice and guinea pigs it has been found that the amount of adjuvant is preferably between 0.01 mg./dose and 1.0 mg./dose but in larger species doses higher than 1.0 mg., generally of up to 10 mg./dose but may be even higher are more appropriate. In any case the dose to be used is one non-toxic to the recipient.

According to the present invention there is further provided:

- a) a method for inducing immunity in non-human animals which comprises administering a vaccine according to the present invention;
- b) a method for the production of a vaccine which comprises the admixture of non-pathogenic antigenic material with a base-rich peptide in which at least 50% of the residues have free amino groups; and
- c) a kit suitable for immunising animals which kit comprises
  - (i) one or more segregated dosages of non-pathogenic antigen material;
  - (ii) a corresponding number of segregated quantities of adjuvant material; each quantity being that required to be mixed with a dose of the antigenic material to produce a vaccine in accordance with claim 1;
  - (iii) the whole being adapted for the extracorporeal preparation of such a vaccine.

The following examples illustrate the present invention:

#### Example 1

A solution containing 0.5 mg. poly - L - lysine (M.W. ca. 44,000) and 2.5 Lf units purified diphtheria toxoid per ml. was made by dissolving the poly - L - lysine in a dilution of diphtheria toxoid in borate succin-

ate buffer pH 7.5. This buffer contained sodium borate  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  0.9% w/v., succinic acid 0.29% w/v. and sodium chloride 0.75 w/v.

- 5 The solution containing polylysine and diphtheria toxoid was injected subcutaneously into groups of five guinea pigs in a volume of 0.2 ml. per guinea pig. Twenty eight days later a second dose of diphtheria toxoid without polylysine was injected. The guinea pigs were bled ten days after the second dose, and the diphtheria antitoxin titre of each individual guinea pig was estimated by the guinea pig intracutaneous test of Romer and Sames (Z. Immun. Forsch., 3, 344, 1909) as modified by Glenny and Llewellyn-Jones (J. Path. Bact., 34, 143, 1931). The geometric mean antitoxin titre of the guinea pigs was 11.5 units/ml. compared with a mean titre of less than 0.001 units/ml. obtained with the same dose of diphtheria toxoid without adjuvant.

#### Example 2

- 25 A solution containing 0.5 mg. poly - L - ornithine (M.W. ca. 45,000) and 2.5 LF units purified diphtheria toxoid was prepared and injected into guinea pigs as described in Example 1.

- 30 The geometric mean antitoxin titre of the guinea pigs injected with the polyornithine diphtheria toxoid solution was 20 units/ml. compared with a mean of less than 0.001 units/ml. obtained with diphtheria toxoid without adjuvant.

#### Example 3

- 35 A dilution of purified diphtheria toxoid, toxoided in the presence of lysine according to the method of Linggood F.V. *et al.*, (Brit. J. exp. Path., 44, 177, 1963) was prepared with borate-succinate buffer to contain 10 Lf units/ml. Poly-L-lysine was dissolved in the same buffer to give a solution containing 1 mg/ml. The two solutions were mixed in equal quantities to yield a single solution containing 5 Lf units of diphtheria toxoid and 0.5 mg polylysine per ml. which was then injected into a group of 5 guinea pigs as described in Example 1. A similar solution of diphtheria toxoid was prepared which contained no polylysine and this was injected similarly into 5 guinea pigs.

- 50 The geometric mean antitoxin titre of the guinea pigs receiving toxoid but no polylysine was 5.1 units/ml. The geometric mean antitoxin titre of the animals receiving toxoid and polylysine was 19.5 units/ml.

#### Example 4

- 60 A dilution of purified tetanus toxoid was prepared with borate-succinate buffer to contain 0.5 Lf units/ml. Poly - L - lysine was dissolved in the same buffer to give a solution containing 1 mg/ml. The two solutions were mixed in equal quantities and injected

into guinea pigs as in Example 3. A similar solution of tetanus toxoid containing no polylysine was also injected into guinea pigs. The serum tetanus antitoxin titres were estimated by the mouse intracutaneous method.

The geometric mean antitoxin titre of the guinea pigs receiving toxoid but no polylysine was less than 0.01 units/ml. The geometric mean antitoxin titre of the animals receiving toxoid and polylysine was 3.3 units/ml.

#### Example 5

Tetanus toxoid adsorbed onto aluminium hydroxide was diluted in physiological saline (0.85% sodium chloride) to give dilutions containing 0.5 and 5.0 Lf units/ml. Poly - L - lysine was dissolved in physiological saline to give a solution containing 1 mg/ml. The dilutions of adsorbed tetanus toxoid were each mixed with equal quantities of the polylysine solution, and also with equal quantities of physiological saline. The solutions were each injected into 5 guinea pigs as described in Example 3.

The geometric mean antitoxin titre of the guinea pigs receiving adsorbed tetanus toxoid without polylysine at the lower dose (0.05 Lf units per guinea pig) was 0.68 units/ml or less, and at the higher dose (0.5 Lf units) 32 units/ml. The corresponding titres for the animals receiving toxoid with polylysine were 9.8 units/ml and 78 units/ml.

#### Example 6

Dilutions of tetanus toxoid adsorbed onto aluminium hydroxide in physiological saline were prepared and mixed with polylysine solution or with further physiological saline as described in Example 5 but to give solutions containing a final concentration of 1, 5 and 20 Lf units, with or without 0.1 mg polylysine per 0.6 ml.

The solutions were injected in a dose of 0.6 ml. once only, subcutaneously, into groups of 10 guinea pigs. The guinea pigs were bled at intervals following injection and the serum tetanus antitoxin titre estimated by the mouse intracutaneous method.

At all intervals the animals receiving polylysine in addition to the toxoid had higher antitoxin titres than those receiving no polylysine. For example, at 6 months following injection those receiving no polylysine had geometric mean titres of 2.0, 6.1 and 10.4 units/ml. for the 1, 5 and 20 Lf unit doses respectively, while those receiving polylysine had corresponding titres of 3.2, 15.3 and 29 units/ml.

#### Example 7

Dilutions of *Cl. welchii* epsilon toxoid were prepared in physiological saline and mixed in equal quantities with either a solution of poly - L - lysine in saline or with saline alone to yield solutions containing a

final concentration of 2 Lf units or 20 Lf units/ml. with or without 0.2 mg. polylysine per ml. The solutions so prepared were injected in a dose of 0.5 ml. into one rabbit each on two successive occasions at an interval of 28 days. Fourteen days after the second injection the rabbits were bled and the serum estimated for antitoxin titre by the rabbit intracutaneous method.

- 5 The titres obtained from the rabbits receiving 1 and 10 Lf units toxoid without polylysine were less than 0.01 units/ml. and 0.05—0.1 units/ml. respectively. The corresponding titres of the rabbits which received toxoid with polylysine were 0.1—0.2 units/ml. and 10—20 units/ml. respectively.

#### Example 8

- 20 Poly - L - lysine was mixed with killed calf pneumonia virus and injected into groups of 8 chicks twice at 21 days interval. Fourteen days after the second dose the birds were bled and the antibody to the virus measured by haemagglutination inhibition.

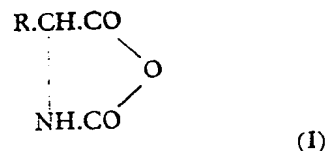
- 25 The geometric mean titre of the birds receiving virus with polylysine was 394, compared with a titre of 84 in birds receiving virus without polylysine.

- 30 The peptides of the invention may be prepared by a number of known methods. Thus, they may be formed from the naturally-occurring L-amino acids, or from their optical isomers the D-amino acids, or from the racemic DL-amino acids. The synthetic peptides are not necessarily products of homogeneous structure, i.e., they do not necessarily consist solely of molecules all having exactly the same number of linked amino acids, but can consist of mixtures of molecules of slightly differing chain length. Nevertheless, by choosing the reaction conditions, they can be made to have any desired mean chain length and they may be made under certain specified conditions from starting materials of any desired chemical purity to give products of such constant characteristics as may be required for pharmaceutical purposes.

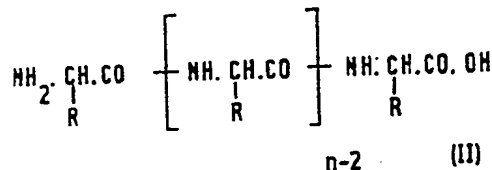
- 45 Whether a polyamino acid peptide is homogeneous or non-homogeneous in structure, depends upon the precise method of synthesis. 50 Peptides of high molecular weight may be prepared by random polymerisation and this method generally gives rise to a non-homogeneous polyamino acid. However, if the synthesis is carried out by the sequential addition of amino acid units then the polyamino acid product will generally be of homogeneous structure. The latter method can usually only be employed in the synthesis of polyamino acids of low molecular weight.

- 60 The peptides of the present invention may, for example, be prepared by the random polymerisation procedure described below:—

An amino acid *N*-carboxy anhydride of formula (I)



where R is the typical group of the amino acid, prepared, for example, by the method described in U.K. patent specification No. 744,988, is polymerised to produce a polyamino acid of formula (II)



where n is the chain length.

The polymerisation reaction is initiated by addition of a component containing hydrogen which may be water, sodium hydroxide, sodium ethoxide, a primary or secondary base such as ethylamine or diethylamine, an amino acid or a peptide.

Where the group R contains a basic group, e.g., in the *N*-carboxy anhydride of lysine, this group has to be suitably protected to prevent its participation in the reaction. A convenient protecting group is benzyloxycarbonyl and this group may be removed by standard methods at the end of the polymerisation reaction.

The value of n in the final product depends on the conditions of polymerisation and in particular on the ratio of the initiator to the carboxy anhydride. In practice, the final product is not a single polymer but a mixture of polymers of various molecular weights. By careful control reaction conditions, the spread of molecular weights about the average molecular weight can be comparatively small. In order to narrow this spread, the final product may, if necessary, be fractionated, for example, by dialysis or chromatography on a molecular sieve.

A method of preparing peptides of known constitution (and therefore of precise molecular weight) is the so-called 'solid phase' synthesis. A polystyrene-2% divinylbenzene resin is chloromethylated to give (P)CH<sub>2</sub>Cl where (P) represents the resin. This is treated with the *N*<sup>+</sup>-tert-butylloxycarbonyl derivative of the amino acid (III)



where BOC is tert-butyloxycarbonyl and R is

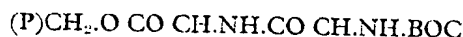
protected by a benzyloxycarbonyl group if it contains a basic group, in the presence of triethylamine to produce (IV)



R

(IV)

- 5 The BOC group is removed by standard methods. The liberated  $\alpha$ -amino group is attached to a second amino acid residue in the form of a compound of formula (III), the condensing agent being dicyclohexylcarbodiimide. This gives (V)



R

R

(V)

- 15 The process is repeated until one has the desired number of  $\alpha$ -amino acid residues in place. The resin is removed by treatment with HBr/trifluoroacetic acid which simultaneously removes the terminal BOC and any benzyloxycarbonyl protecting groups. The product is the trifluoroacetate of the poly-amino acid, and this may be dissolved in acetic acid and the polyamino acid hydrochloride precipitated by addition of an HCl/acetic acid mixture.

20 The following examples illustrate the methods for preparing polyamino acids.

#### Example A

25 Preparation of poly-L-lysine hydrobromide (average mol. wt. 20,000)

- 30 To a solution of N<sup>ε</sup>-benzyloxycarbonyl L-lysine N<sup>α</sup>-carboxyanhydride (20 g.) in dry dioxan (200 ml.) was added 12.6 ml. of a solution of ammonia in dioxan (0.663 mg. NH<sub>3</sub>/ml.). After stirring for one hour at ambient temperature, the mixture was heated and maintained at 100°C (oil bath temp.) for 42 hours. The cloudy solution was cooled and poured into dry petroleum ether (500 ml. b.p. 60–80°C) with stirring. The solvent was decanted and the precipitated solid was washed by stirring with a further 400 ml. petroleum ether. The solid was filtered and dried *in vacuo*. Yield 15.9 g.

- 40 Poly N<sup>ε</sup>-benzyloxycarbonyl L-lysine (15.9 g.) was dissolved in trifluoroacetic acid (150 ml.) and HBr passed through the solution for 45 minutes, with stirring. The excess HBr was removed in a stream of nitrogen and ether (250 ml.) added. The precipitates solid was filtered, washed with dry ether and dried *in vacuo*. The precipitates solid was filtered, washed with dry ether and dried *in vacuo*. The somewhat hygroscopic solid was dissolved in water (50 ml.) and dialysed (24/32 Visking tubing) against three changes

each of 500 ml. of distilled water over a period of 24 hours. The contents of the tube were filtered and freeze-dried to give poly L-lysine hydrobromide (8 g.). The average molecular weight of the polymer was found to be 20,000 and there was no evidence of a high degree of polydispersity.

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#### Example B

Preparation of poly L- $\alpha,\gamma$ -diaminobutyric acid trifluoroacetate

To a solution of N<sup>ε</sup>-benzyloxycarbonyl L- $\alpha,\gamma$ -diaminobutyric acid N<sup>α</sup>-carboxy anhydride (7 g.) in dry dimethylformamide (100 ml.) was added 0.55 ml. of a 10% v/v solution of diethylamine in dimethylformamide. After stirring at ambient temperature for 2 days, the solution was diluted with water (150 ml.), the solid filtered, washed with water and dried *in vacuo* (6.5 g.).

The above benzyloxycarbonyl polymer (6.5 g.) was heated at 60°C for 1½ hours with trifluoroacetic acid (50 ml.). The solvent was removed under reduced pressure and the residue triturated with ether. The solid was filtered, washed with ether and dried *in vacuo* (4.65 g.). The solid was dissolved in water (60 ml.) and dialysed (24/32 Visking tubing) against three changes each of 500 ml. distilled water for five hours. The contents of the tubing were filtered and freeze-dried to give poly L- $\alpha,\gamma$ -diaminobutyric acid trifluoroacetate (3.5 g.).

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#### Example C

Preparation of poly L-norarginine

Poly L- $\alpha,\gamma$ -diaminobutyric acid trifluoroacetate (2 g.) was dissolved in ammonia solution (18 ml.; specific gravity 0.880) and S-methylisothiurea sulphate (4.8 g.) added, and the mixture stirred at ambient temperature for 24 hours. The liquid was decanted from the precipitated gum which was then washed with water (20 ml.). The gum was dissolved in warm 2N-H<sub>2</sub>SO<sub>4</sub> (200 ml.) and

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flavianic acid (7.5 g.) in water (15 ml.) added. After refrigeration for several hours the yellow precipitated was filtered, washed with a saturated solution of flavianic acid in water and then with hacetone. The solid was dissolved in warm 2N-H<sub>2</sub>SO<sub>4</sub> (300 ml.) and after cooling

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was extracted with three portions of 1-butanol (100 ml.) to remove flavianic acid. The colourless solution was adjusted to pH 5 by the addition of a hot saturated solution of baryta, the barium sulphate filtered and the filtrate concentrated *in vacuo*. The residual gum was dissolved in water (50 ml.) and freeze-dried to give poly L-norarginine sulphate (0.95 g.) as a white, somewhat hygroscopic, solid.

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### Example D

#### Preparation of polylysines by solid phase synthesis

Chloromethylated polystyrene-2%, divinyl-  
5 benzene resin (0.84 millimoles Cl/g.) (40 g.)  
and  $N^{\alpha}$ -tert-butylloxycarbonyl- $N^{\epsilon}$ -benzyloxycarbonyl L-lysine (12.8 g.) (and triethylamine (4.55 ml.) in absolute ethanol (300 ml.) were gently refluxed with stirring for 48 hours. The resin was filtered and washed with ethanol (250 ml.), water (250 ml.) and methanol (500 ml.) and dried *in vacuo*. Analysis of the resin showed that 0.27 millimoles of the amino acid/g. resin had been introduced.

The resin (10 g.) was placed in the reaction vessel designed for solid phase syntheses and four cycles of the reactions (a) removal of the  $N^{\epsilon}$ -BOC residue with  $NHCl$ /acetic  
20 acid, (b) neutralisation of the hydrochloride with  $NEt_3$  in chloroform, (c) peptide formation by addition of  $N^{\alpha}$ -tert-butylloxycarbonyl- $N^{\epsilon}$ -benzyloxycarbonyl-L-lysine (4.1 g.) and dicyclohexylcarbodiimide (2.22 g.) were complete. The terminal  $N^{\epsilon}$ -BOC group was removed with  $NHCl$ /HAc

and the resin (11.86 g.) was dried in a stream of nitrogen.

A portion of the resin (7.1 g.) was removed,  
30 suspended in trifluoroacetic acid and treated with  $HBr$  for 90 minutes. The mixture was filtered, washed with trifluoroacetic acid and the filtrate concentrated *in vacuo*. The residue was triturated with ether, the solid filtered,

washed with ether dissolved in water and freeze-dried, to give  $(Lys)_n$  trifluoroacetate (0.90 g.). The trifluoroacetate was dissolved in warm water (25 ml.),  $NHCl$ /acetic acid

(5 ml.) added and the precipitated  $(Lys)_n$  hydrochloride (0.70 g.), filtered, washed with ether, and dried.

The remaining portion of the resin used, by an additional five cycles of the reaction described above, to prepare  $(Lys)_n$  hydrochloride.

$(Lys)_n$  hydrochloride and  $(Lys)_n$  hydrochloride were also prepared in a similar manner.

### Example E

#### Preparation of polyhomoarginine sulphate

$(Lys)_n$  hydrobromide (2 g.), 0.880 ammonia (18 ml.) and *S*-methylisothiurea (4.8 g.) were stirred at ambient temperature for 48 hours. The supernatant liquid was decanted from the precipitated gum, which was washed with a little water and dissolved in 2*N* sulphuric

acid (200 ml.). Flavianic acid (10 g.) was added and the precipitate filtered, washed with water and with acetone. The yellow solid was dissolved in warm 2*N* sulphuric acid

(200 ml.) and the flavianic acid extracted with 1-butanol ( $3 \times 100$  ml.). Hot baryta solution was added to pH 5.0. The barium sulphate was filtered, the solution concentrated *in vacuo*, the residue dissolved in water (30 ml.) and freeze dried, to give (homoarginine) $_n$  sulphate (1.1 g.) as a somewhat hygroscopic solid.

### Example F

#### The heteropolymer

70 Pel—DAB—DAB—D—Leu—Leu—DAB—DAB—DAB—OH.

where DAB represents diaminobutyric acid and Pel represents pelargonyl, was prepared by the solid phase synthesis described below.

Chloromethylated polystyrene-2%, divinyl-  
75 benzene resin (0.82 millimoles Cl/g.) (49 g.),  $N^{\alpha}$ -tert-butylloxycarbonyl- $N^{\epsilon}$ -tosyl L- $\alpha,\gamma$ -diaminobutyric acid (4.07 g.) and triethylamine (5.58 ml.) were refluxed with stirring for 66 hours. The resin was filtered and washed with ethanol (500 ml.), water (500 ml.) and methanol (500 ml.), and dried *in vacuo*. Analysis of the resin showed that 0.39 millimoles of the amino acid/g. resin had been introduced.

85 The resin (10 g.) was placed in the reaction vessel and the following amino acids were sequentially coupled by the general procedure described in Example D, using four equivalents of the amino acid derivative and  
90 four equivalents of dicyclohexylcarbodiimide,

BOC.DAB, BOC.DAB,

Tos Tos

BOC.Leu, BOC.D-Leu,

BOC.DAB and Pel.DAB.

Tos Cbz

On completion of the final coupling the resin was suspended in trifluoroacetic acid and treated with gaseous  $HBr$  for 90 minutes. The mixture was filtered, washed with trifluoroacetic acid and the filtrate concentrated *in vacuo*. The residue was triturated with ether, the solid filtered, washed with ether and dried *in vacuo*. The solid (3.8 g.) was dissolved in dimethylformamide (5 ml.), pyridine (0.25 ml.) was added and the mixture diluted with water (60 ml.). The

precipitated solid (3.54 g.) was filtered, washed with water and dried *in vacuo*.

Abbreviations:

- 5           Tos = *p*-toluenesulphonyl  
            BOC = *tert*-butoxycarbonyl  
            Cbz = benzyloxycarbonyl

WHAT WE CLAIM IS:—

1. A vaccine comprising non-pathogenic antigenic material together with a base-rich peptide in which at least 50% of the residues have free amino groups, which is present in an amount sufficient to produce an adjuvant effect.
2. A kit for immunising animals which kit comprises:
- (i) one or more segregated dosages of non-pathogenic antigenic material;
- (ii) a corresponding number of segregated quantities of adjuvant material, each quantity being that required to be mixed with a dose of the antigenic material to produce a vaccine in accordance with claim 1;
- (iii) the whole being adapted for the extra-corporeal preparation of such a vaccine.
3. A vaccine or kit according to any one of the preceding claims wherein, in the peptide, each amino acid residue has a free amino group.
4. A vaccine or kit according to claims 2 or 3, wherein the peptide is selected from polylysine, polyornithine, polyarginine and polydiaminobutyric acid.
5. A vaccine or kit according to claim 4 wherein the peptide is poly - L - lysine.
6. A vaccine or kit according to any one of the preceding claims wherein the peptide

has a molecular weight in the range from 6,000 to 50,000.

7. A vaccine or kit according to any one of claims 1 to 5, wherein the peptide has a chain length of 5 to 30 units.

8. A vaccine or kit according to any one of the preceding claims 1 to 5, wherein the antigenic material contains diphtheria toxoid.

9. A vaccine or kit according to any one of claims 1 to 7, wherein the antigenic material contains tetanus toxoid.

10. A vaccine or kit according to either of claims 8 and 9, wherein the toxoid is adsorbed onto aluminium hydroxide.

11. A vaccine or kit according to any of claims 1 to 7, wherein the antigenic material contains *Clostridium welchii* epsilon toxoid.

12. A method for the production of a vaccine, which comprises the admixture of non-pathogenic antigenic material with a peptide as defined in any one of claims 1—6.

13. A method according to claim 12, wherein the peptide is defined according to any one of claims 2 to 7.

14. A method according to claim 12 or 13, wherein the antigenic material is defined according to any one of claims 8 to 11.

15. A method for inducing immunity in non-human animals which comprises administering a vaccine according to claim 1 or any one of claims 3 to 11.

16. A vaccine according to claim 1, substantially as described herein.

17. A method for the production of a vaccine according to claim 12, substantially as described herein.

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Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1972.  
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

